Studying the hypoglycemic and the antibacterial activity of various plant extract of *Urtica dioica*

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ABSTRACT:-

The present study was designed to investigate the effects of methanolic and water (hot,cold) extracts of *urtica dioica* plant in blood glucose level of aloxan induced diabetic laboratory rats . And also to study the antibacterial activity of these extracts against different groups of gram positive and negative bacterial species . the present study showed the presence of significant $p \leq 0.05$hypoglycemic activity of blood glucose in the group treated with the hot methanolic extract compared with control and (hot ,cold) water extracts groups.The results showed significant $p \leq 0.05$ antibacterial activity in the hot methanolic extract against most the gram positive and negative bacterial species compared with standard antibiotics and (hot ,cold) water extracts . The result confirm that hot methanolic extract of *urtica dioica* has more activity than the water extracts.

Introduction

Diabetes mellitus is one of the most common metabolic diseases in the world. , In fact, Diabetes Mellitus is a clinical syndrome, characterized by hyperglycemia caused by a relative or absolute deficiency of insulin at the cellular level. It is the most common endocrine disorder, affecting mankind all over the world[(1)]. Traditional preparations from plant sources are widely used almost everywhere in the world to treat this disease. Therefore, plant materials are considered to be the alternative sources, for discovering new leads for anti-diabetic agents[(2)]. More than 800 species have been reported to display anti-diabetic effects, but few of them have been investigated scientifically. However, the studies on antidiabetic plants are relatively recent and has begun to evolve in the last few years.[(3)].As the knowledge of heterogeneity of this disorder has increased, it is needed to look for more efficacious agents with lesser side effects. Moreover, the existing drugs do not modify the course of diabetic complications [(4)] .Plants used in folk medicine to treat diabetes mellitus

represent a viable alternative for the control of this disease[(5)]. *Urtica dioica L. and Urtica urens L.*, (stinging nettles) have a long history of use

in folkloric and science based herbal medicine. Traditionally used as a nutritive and "blood cleanser" or alterative agent, a substantial pharmacological and clinical literature supports its use for arthritic and allergic conditions (leaf/herb) and improving urological symptoms of benign prostatic hyperplasia (root)[(6,7)] . *Urtica dioica* (U. dioica), an annual and perennial herb of family *Urticaceae* is commonly known as medical herb for a long time in the world. This herb is known for its anti- inflammationtory activity [(8,9)]. There have been also other reports indicating the benefits of using the extract of the leaves or other parts for the use in different conditions, i.e., diabetes [(10,11,12, 13)] as well as other disorders like prostatic hyperplasia [(14).] rheumatoid arthritis, hypertension and allergic rhinitis [(15)]. Uterine hemorrhage and coetaneous eruption [(16)].The herbs

are used to treat stomach ache in Turkish folk medicine and is used against liver insufficiency[17,18]. Although, there are some reports regarding the hypoglycemic and antimicrobial activity of *U.dioica* in folk medicine but, in other hand, several investigations have detected hyperglycemic activity of this herb [19] and noticeable antibacterial activity .Therefore, this study was done to evaluate the effects of different extracts of *U. dioica* in blood glucose level in laboratory rat and investigate the antibacterial activity of these extracts against some gram positive and negative bacterial species.

Methods

Collection and Identification

The plant *Urtica dioica* was bought from a local market as Dried herbs and identified by taxonomist Dr. Ali Aboud Shareef biology department college of Education University of Basrah . The leaf of the plant was grounded and 40 gms of the sieved powder was weighed accurately and subjected to extraction in a soxhlet apparatus using methanol and water as a solvent extract , 2 types of extraction (hot and cold) are used successively for each solvent. Before extraction with the next solvent the powder was air dried to remove the adhering solvent. The extract obtained was filtered, concentrated in rotary flash evaporator and dried in a vacuum oven.[20]

1. **Animals :**

75 Adult Male albino rats weighing 150-200 g were used in the present study. All rats were kept in the animal house in college of pharmacy university of kufa at room temperature .They were fed with standard rat pellet diet and provided water ad libitum.The animals were treated with 250 mg /kg and this dose was selected after a series of primary experiments.

3-Alloxan-induced diabetes:

The rats weighing 150-200 g were allowed to fast for 24 hours prior to experimentation and rendered diabetic by a single dose of intraperitoneal injection of alloxan 150 mg/kg body weight dissolved in normal saline [21] After 48 hours of injection of alloxan, diabetes was confirmed by testing blood sugar. The level more than 200 mg/dl were selected for the further study. then the animal were divided into the following groups each with 15 rats and treated the plant extract orally using stomach tube.[22] .

Group 1: rats treated with 250 mg /kg of cold methanolic extract mg/kg.

Group 2: rats treated with 250 mg /kg of hot methanol extract.

Group 3: rats treated with 250 mg /kg of cold water extract.

Group 4: rats treated with 250 mg /kg of hot water extract.

Group 5: rats treated with normal saline as control group

Collection of Blood Sample:-

Blood samples were collected directly by heart puncture at zero (as fasting and 2, 4,6,8 hrs after oral administration) and Prior to killing. The animal must be food deprived and drinking water

was then replaced by glucose solution 20% to preveut hypoglycemia.Blood glucose levels were assayed by using enzymatic colorimetric test (GOD-PAP) using a standard kit [23]

3- Antimicrobial activity (antibacterial testing).

Seven species of bacteria *E.coli, Pseudomonas aerogenosa ,Klebsiella,Staphylococus aureus,Sallmonella,Streptococcus,Proteus* were used in this study obtained from the Department of Microbiology, colloge of Medicine,kufa university.Bacterial species were identified according to[24],and maintained on nutrient agar plates and recovered for testing by sub-culturing in nutrient broth for 24hrs[25].the antimicrobial activity tests were then carried out by agar diffusion assay [26], wells (6mm diameter)were aseptically punched on each agar plate using sterile cork-borer ,2-4 colonies of the tested bacteria were inoculated in water and these inoculums was swabbed (for each species),using steril swab on the surface of above punched nutrient agar plates,a fixed volumes of the plant extracts in the concentrations (50µg/ml,100µg/ml)was then introduced into wells,and then incubated at 37C°.Cefuroxime(30mg per disc),gentamycin (40 mg per disc)were used as reference standards which as recommended by the National Committee for clinical laboratory standards.Antimicrobial activity was evaluated by measuring the inhibition zones diameter after 24 hrs of incubation (each assay in this experiment was repeated triple times).

Statistical Analysis:

The Statistical analysis of the results were performed by using the student's t-test (paired) or ANOVA (analysis of variance). The limit of significance was set at $p \leq 0.05$. The Data from the experiments were analyzed using the Statistical (SPSS) software for windows version 12 (SPSS Inc.).

Results

The present result showed no significant differences between hot and cold water extract and control group figure (1) the statistics showed significant $p \leq 0.05$ decreasing in blood glucose level in the groups treated hot and cold methanol extracts compared with control group figure(2) .While.the results between the periods of treatment showed significant $p \leq 0.05$ decreasing in blood glucose level in the group treated with hot methanol extract in the period (4,6 and 8hr)compared with (2hr and zero) period and there is significant $p \leq 0.05$ decreasing between the period (4hr,6hr,8hr) fig(2).while the results showed no significant differences in blood glucose level in the periods(zero,2hr). Also the result in the group treated with cold methanol extract showed significant $p \leq 0.05$ decreasing in blood glucose level in the period (6hr and 8hr)compared with (2hr,4 hr and zero). And there is significant differences between the period (6hr and 8hr) while there is no significant decreasing between these periods(2hr,4 hr and zero)fig(2) the result in the group treated with hot and cold water extract showed no significant differences between the periods of the treatment fig(1).

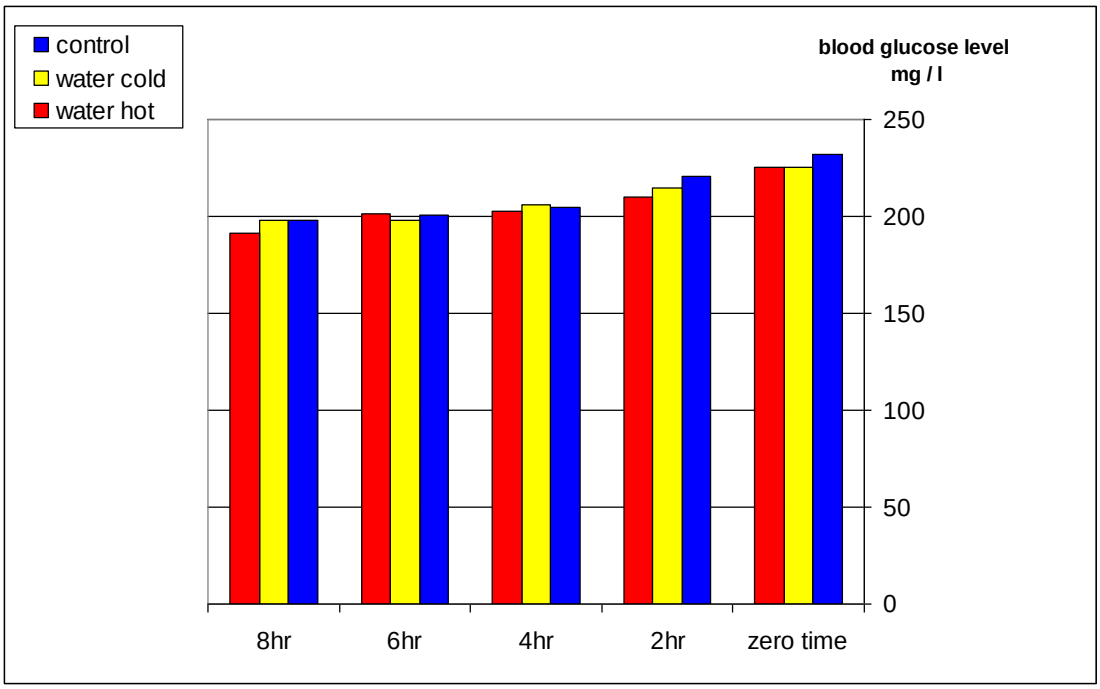

Figure (1) effect of hot and cold water extracts in blood glucose level mg/l in laboratory rats n=5

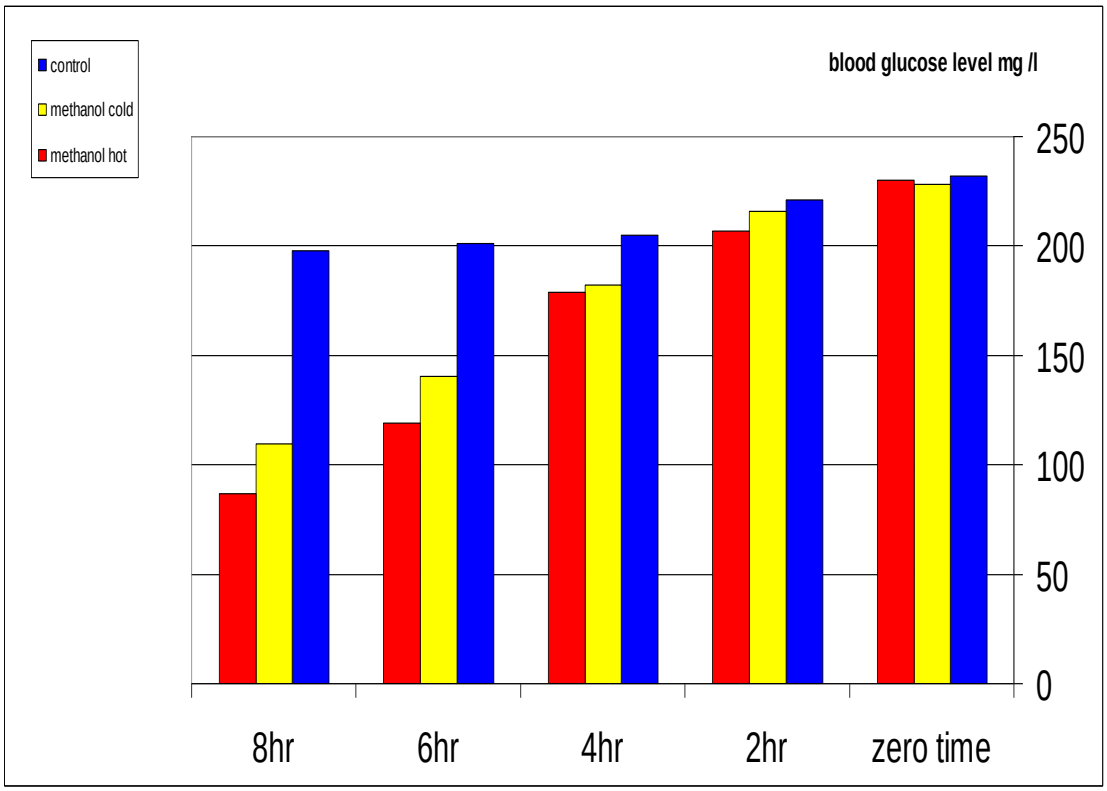

Figure (2) effect of hot and cold methanol extracts in blood glucose level mg/l in laboratory rats n=5

Antibacterial activity

The statically analysis showed that there significant p≤ 0.05 increasing in antibacterial activity in control standard antibiotics compared with cold and hot water extract specially in cefuroxime antibiotic against most of the tested bacteria . except in *streptococcus and salmonella* figure(3). the result showed there is significant p≤0.05 increasing in the antibacterial activity in both concentration of hot water extracts compared with standard antibiotics figure(4).While The result in methanolic extracts showed significant p≤0.05 in increasing in antibacterial activity in both cold and hot extract in(50,100 mg) compared with control standard antibiotics figure(5).The result also showed highly significant p≤0.05 in the antibacterial activity in hot methanolic extract compared with cold extract specially against *Streptococcus, pseudomonas and proteus* figure(5,6) . the comparison between the extracts in the antibacterial activity showed significant p≤0.05 increasing in hot extracts compared with other extracts against all tested bacterial species specially in 100mg treatment of the extracts figure(7,8).

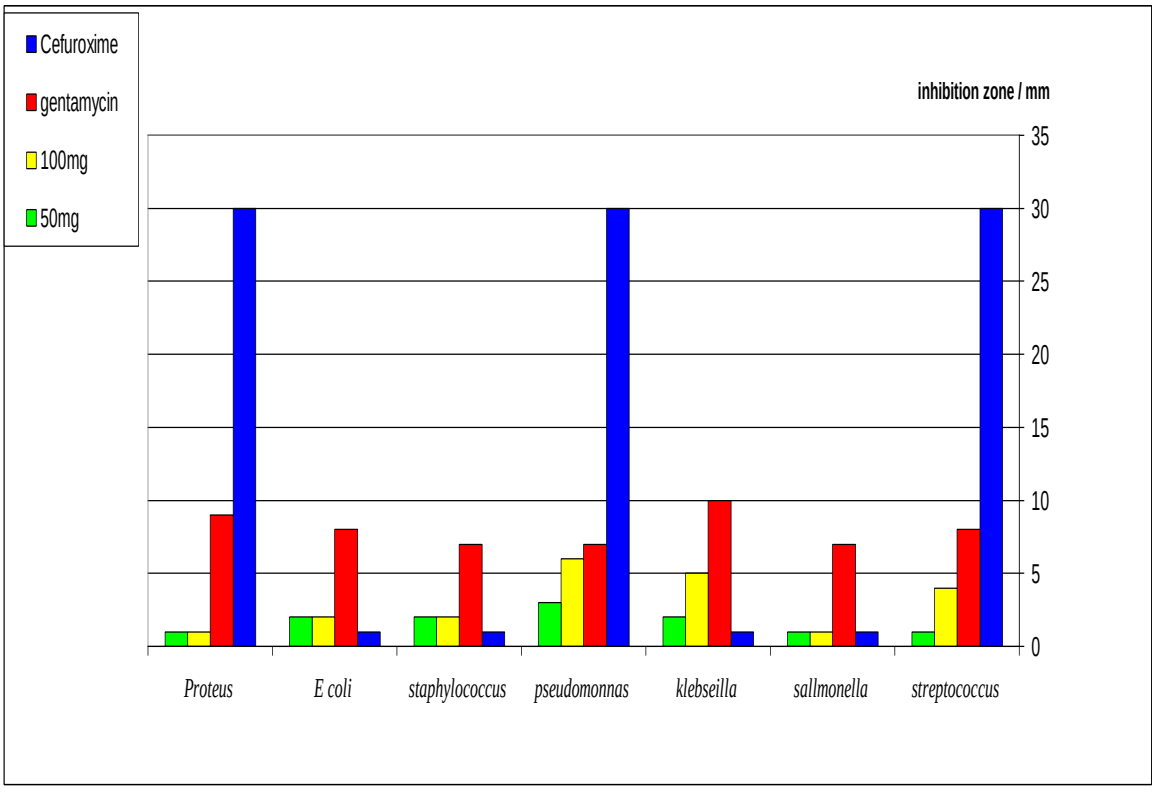

Figure (3) Antibacterial activity of cold water extract against tested bacteria

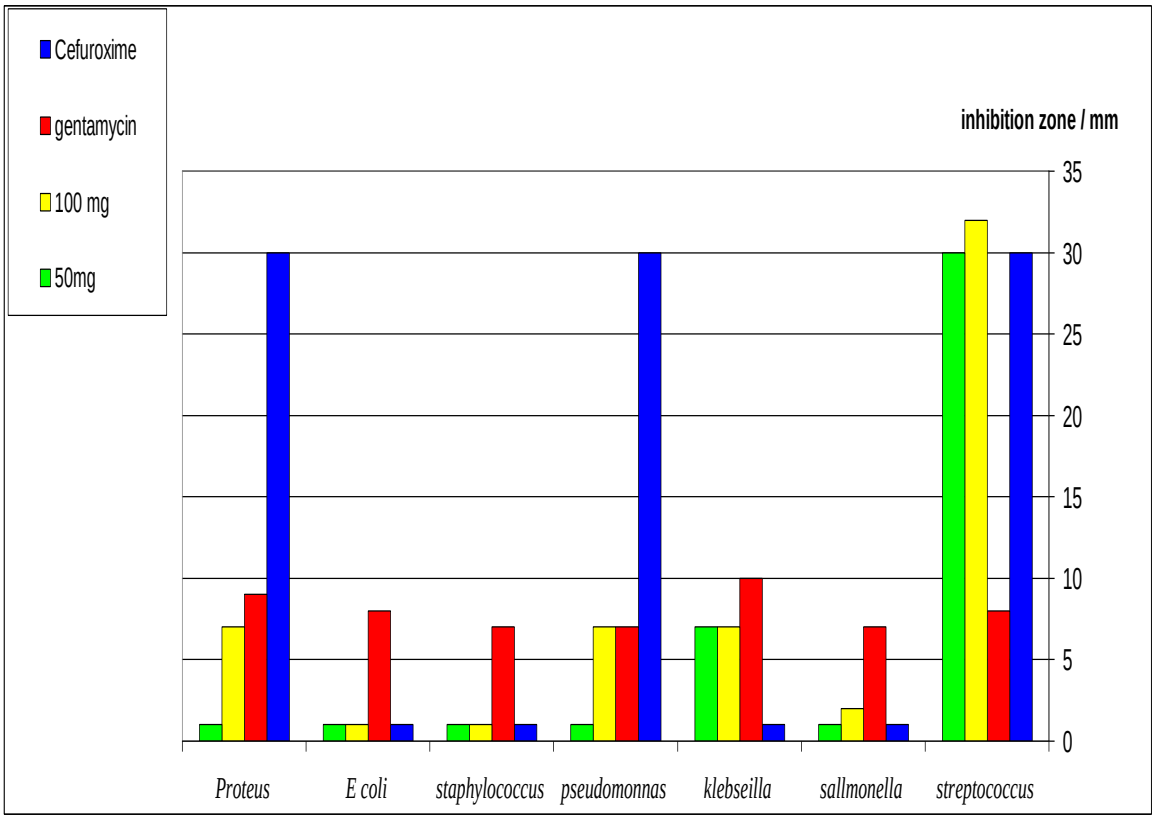

Figure (4)Antibacterial activity of hot water extract against tested bacteria

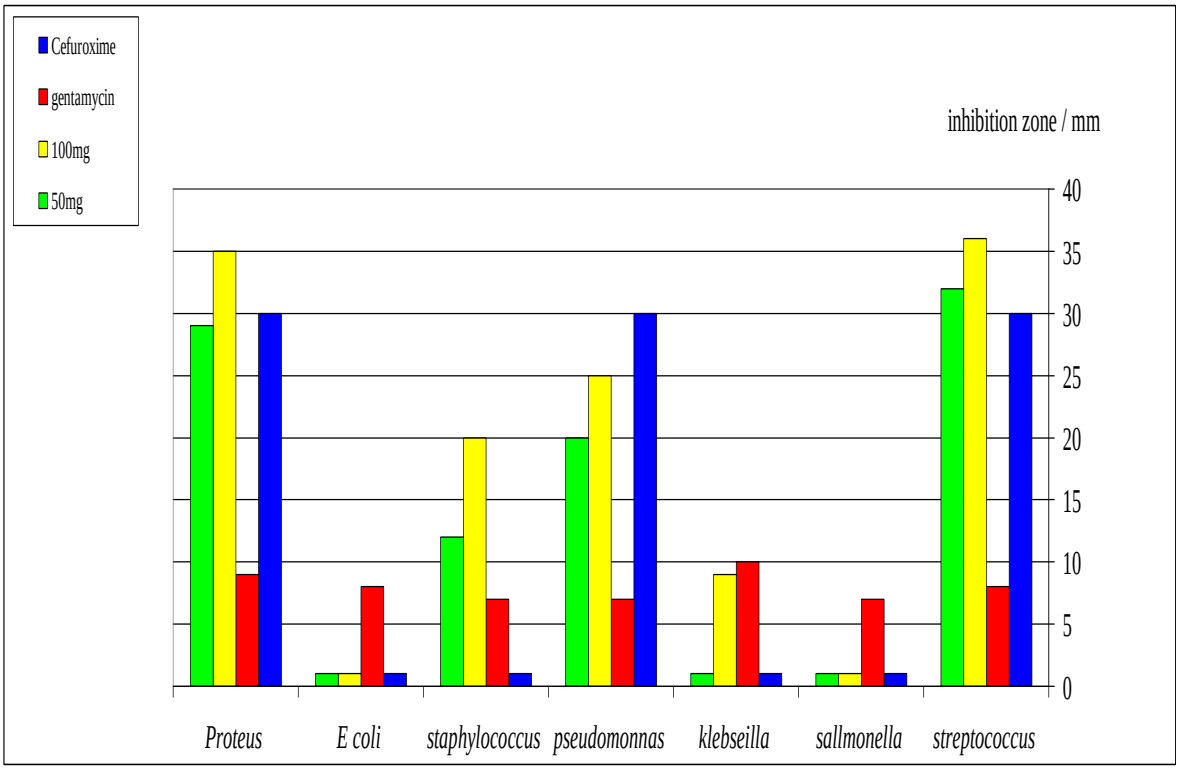

Figure (5) Antibacterial activity of cold metanol extract against tested bacteria

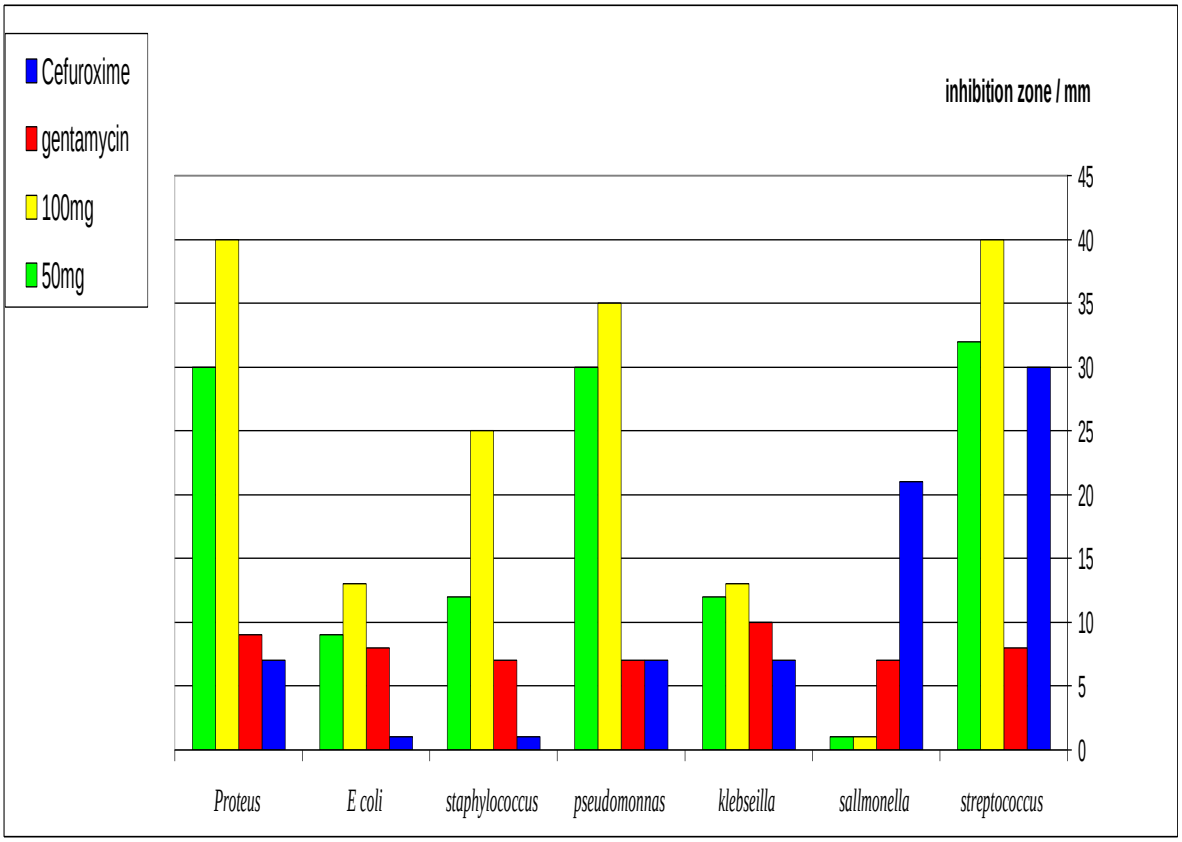

Figure (6) Antibacterial activity of hot methanol extract against tested bacteria

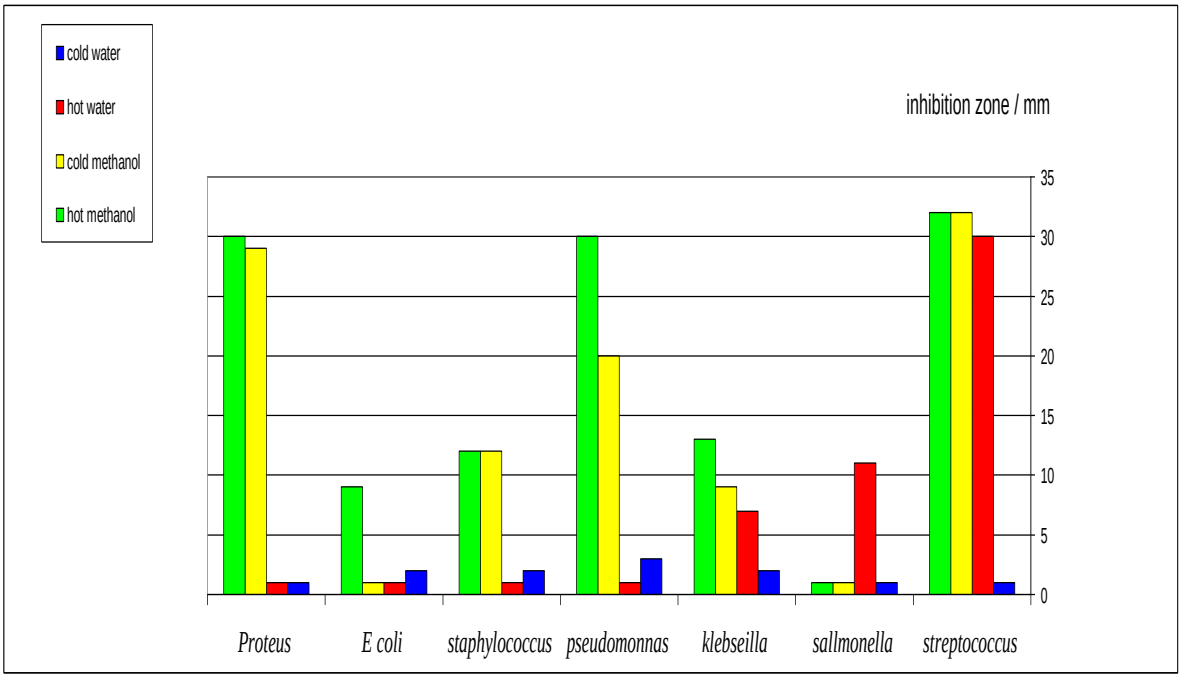

Figure (7) Comparison in the antibacterial activity between methanolic and water (hot,cold) extracts in 50mg/ml against tested bacteria

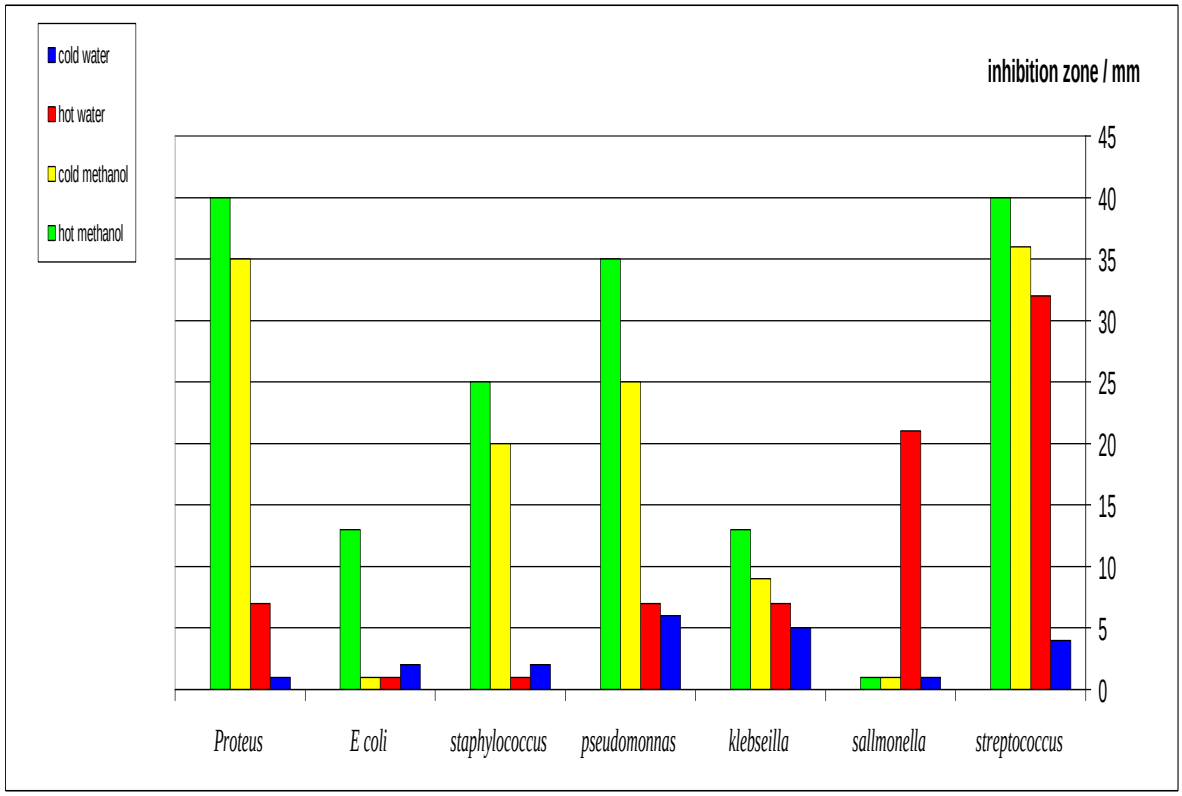

Figure (8) Comparison between the antibacterial activity between methanolic and water (hot,cold) extracts in 100mg/ml against tested bacteria.

Discussion

The results of this study showed that *utrica dioica* plant extracts showed hypoglycemic activity. The findings of this study are similar to other studies which is showed that the administration of the *U. dioica* leaves before induction of diabetes in animals can increasing proliferation of β□-cells and deceasing of blood glucose concentration in 60%of Rats. [,27,28])another studies founds that a fraction from *U. dioica* was a potent stimulator of insulin release of β□-cells[29] .the hypoglycemic activity of *U. dioica* may be due to decrease of glucose transport from small intestine [15] the reduction in blood glucose level following the administration of *U. dioica* extract can be attributed to increase activity of ACC as glucose sensor for insulin secretion and NDPK that involves as an energy metabolism of the cell. In this regard, maybe U. dioica extract causes rearrangement of hepatocytes and increase in activity of these enzymes..[30] on the other hand the results in fig(5) showed that water extract in both hot and cold have non significant hypoglycemic activity compared with methanolic extracts these result was agreed with other studies which concluded that *urtica dioica* extract have not shown the hypoglycemic activity of aqueous extract of.[31] generally Most of the included studies showed that *urtica dioica* can significantly reduced blood sugar researchers have proposed several mechanisms for this process. possible effect of *urtica dioica* could be categorized in to two groups of pancreatic and extrapancreatic regarding to the pancreatic effects they have been suggested that utrica enhances the secretagogue function of islets of langerhance and it is apotent stimulator of insulin release from β□ cells [32].The extrapancreatic suggest that *utrica dioica* affects the glucose homeostasis which include inhibition of intestinal absorption of glucose other studies suggest

that utrica dioica has inhibitory effects on alfa amylase activities in dose dependent manner and forming a unique glucose permeable pore to facilitate glucose uptake[33].

Antibacterial activity

It appears from the present result that (*Urtica dioica*) have antibacterial activity and this result agree with [34,35] who founds that *Urtica dioica* have noticeable antibacterial activity against *Streptococcus pyogenes*, *Staphylococcus aureus* and *Staphylococcus epidermidis* .on the other hand the antibacterial activity in *Urtica dioica* may be due to presence of fatty acids in their composition [36] they suggest that the fatty acids in the *U dioica* showed antibacterial activity . The anti bacterial activity of *Urtica dioica* may be due to the presence of phenolic compound in its constituents ·and this suggetion agree with [37] who founds that a Plant phenolics constitute one of the major groups of compounds responsible for antioxidant behavior, as well as for antimicrobial effects. the result in the figure(10) showed that methanolic extract showed significant inhibitory zone compared with water extract these result disagree with[38] whom suggest that the water extract has antibacterial activity. but in general methanol extract exhibited antibacterial activity in both hot and cold. Hence, it can be considered that methanol is a good solvent for the extraction of various active compounds present although specificity in solvent could be exhibited. [,39]. Generally there a lot of factors effect antibacterial activity. So,the bacterial inhibition can vary with the plant extract, the solvent used for extraction, and the tested organism

Conclusions

Our study indicate that the methanolic extract of *urtica dioica* has potential antibacterial activity against some bacterial species .SoThe use of the biologically active compounds from this plant could represent a natural alternative source to antibiotics

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دراسة الفعالية الخافضة للسكروالضد بكتيرية لمستخلصات متنوعة لنبات القريص *Urtica dioica*

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الملخص:-

صممت الدراسة الحالية للتحري عن تأثير المستخلص الكحولي والمائي الحار والبارد لنبات القريص *urtica dioica* في مستوى كلوكوز الدم للجرذان المختبريةالمعاملة بالالوكسان المستحث للسكري فيها وكذلك لدراسة الفعالية الضد بكتيرية لهذه المستخلصات ضد مجموعة من الانواع البكتيرية السالبة والموجبة لصبغة كرام .أظهرت الدراسة الحالية وجود فعالية معنوية$p\leq0.05$ لخفض مستوى كلوكوز الدم وخاصة في المجموعة المعاملة في المستخلص الميثانولي الحار والبارد مقارنة بمجموعة السيطرة والمجاميع المعاملة بالمستخلص المائي الحار والبارد.كذلك اظهرت النتائج وجود فعالية معنوية $p\leq0.05$ ضد ميكروبية للمستخلص الميثانولي الحار ضد اغلب الانواع البكتيرية السالبة والموجبة لصبغة كرام مقارنة بالمضاد القياسي و المستخلصات المائية الحارة والباردة. أثبتت النتائج إن المستخلص الميثانولي اكثر فعالية من المستخلصات المائية.